trophils could switch cell fates, or possibly even revert to the less-differentiated CMP state.

The work of Laslo et al. (2006) represents a significant advance in understanding the molecular mechanisms that regulate CMP differentiation and provides further evidence for the ubiquity of positive feedback in the regulation of cellular decisions and memory. Although it is not the first molecular characterization of mutual corepression in the context of hematopoiesis (Cantor and Orkin, 2001), it is also unlikely to be the last. Irreversible resolution of lineage priming appears to be a common feature of blood cell differentiation and may in fact be a general feature of other developmental processes as well.

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A Transcriptional Logic for Nuclear Reprogramming

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Limitations on a differentiated cell's pluripotency can be erased by nuclear transfer or by fusion with embryonic stem cells, but attempts to recapitulate this process of nuclear reprogramming by molecular means have failed. In this issue of *Cell*, Takahashi and Yamanaka (2006) take a rational approach to identifying a suite of embryonic transcription factors whose overexpression restores pluripotency to adult somatic cells.

The phenomenon of nuclear reprogramming was first demonstrated in the context of somatic cell nuclear transfer experiments. These experiments showed that the developmental state of a nucleus from an adult somatic cell can be reprogrammed upon its transfer into an unfertilized oocyte. Such a strategy can result in the generation of cloned embryos with the potential to develop into another entire animal, such as Dolly the sheep (Wilmut et al., 1997). Although cloning experiments were, and still are, inefficient, they provide definitive proof that pluripotency can be restored to the nucleus of a terminally differentiated cell. Subsequently, cell fusion experiments in which adult somatic cells are fused with mouse embryonic germ cells, mouse embryonic stem (ES) cells, or human ES cells have shown that these pluripotent cells also harbor reprogramming activities (Cowan et al., 2005; Tada et al., 2001). These findings demonstrate the biological reality of nuclear reprogramming, yet the nature and identity of the molecules in an oocyte or pluripotent cell that constitute this activity have remained elusive.

In this issue of *Cell*, Takahashi and Yamanaka (2006) take a significant step toward delineating the minimal set of factors required to confer the developmental potential of an ES cell onto a terminally differentiated somatic cell. Leveraging the knowledge that ES cells have reprogramming capabilities, the authors reasoned that forcing the expression of ES cell-specific genes, particularly transcription factors, in somatic cells might induce them to take on a more embryonic character. In order to assay for reprogramming, mouse fibroblasts were generated that

harbored a drug selection cassette under the control of a promoter active only in ES cells (*Fbx15*). Both mouse embryonic fibroblasts and fibroblasts from the tail tips of adult mice (MEFs and TTFs, respectively) were subjected to this reprogramming strategy. The authors deduced that nuclear reprogramming had taken place if these fibroblasts expressed β-galactosidase activity and became resistant to high concentrations of neomycin (Figure 1A).

To induce reprogramming, the authors cotransduced the target fibroblasts with retroviral vectors, each carrying a unique cDNA encoding a candidate reprogramming molecule. In total, retroviral vectors encoding 24 genes previously implicated in the biology of ES cells were tested. These genes included some known to be involved in the process of self-renewal (*Oct3/4*, *Sox2*, and *Nanog*), those observed by recent in silico studies to be upregulated specifically in ES cells (Mitsui et al., 2003), and some more commonly associated with transformation that have also been implicated in the maintenance of ES cell pluripotency (*c-Myc*, *Eras*, and *Klf4*). The transduction of any one of these factors alone was insufficient to induce expression of the embryonic reporter. Remarkably, however, simultaneous transduction with all 24 cDNA-encoding retroviruses followed by antibiotic selection resulted in the appearance of drug-resistant colonies that had the morphology and growth characteristics of ES cells.

By cleverly repeating this experiment with pools of retroviruses that lacked just one of the 24 candidate genes, Takahashi and Yamanaka (2006) identified a set of ten cDNAs that when introduced together into their Fbx15:β-geo reporter fibroblasts could induce the formation of ES cell-like colonies. The resulting ES-like cells were termed "iPS-MEF10" cells, short for induced pluripotent stem cells from MEFs by 10 factors. By reiterating this approach, the authors ultimately narrowed the pool of cDNAs required to recover iPS cells to just four: *Oct3/4*,

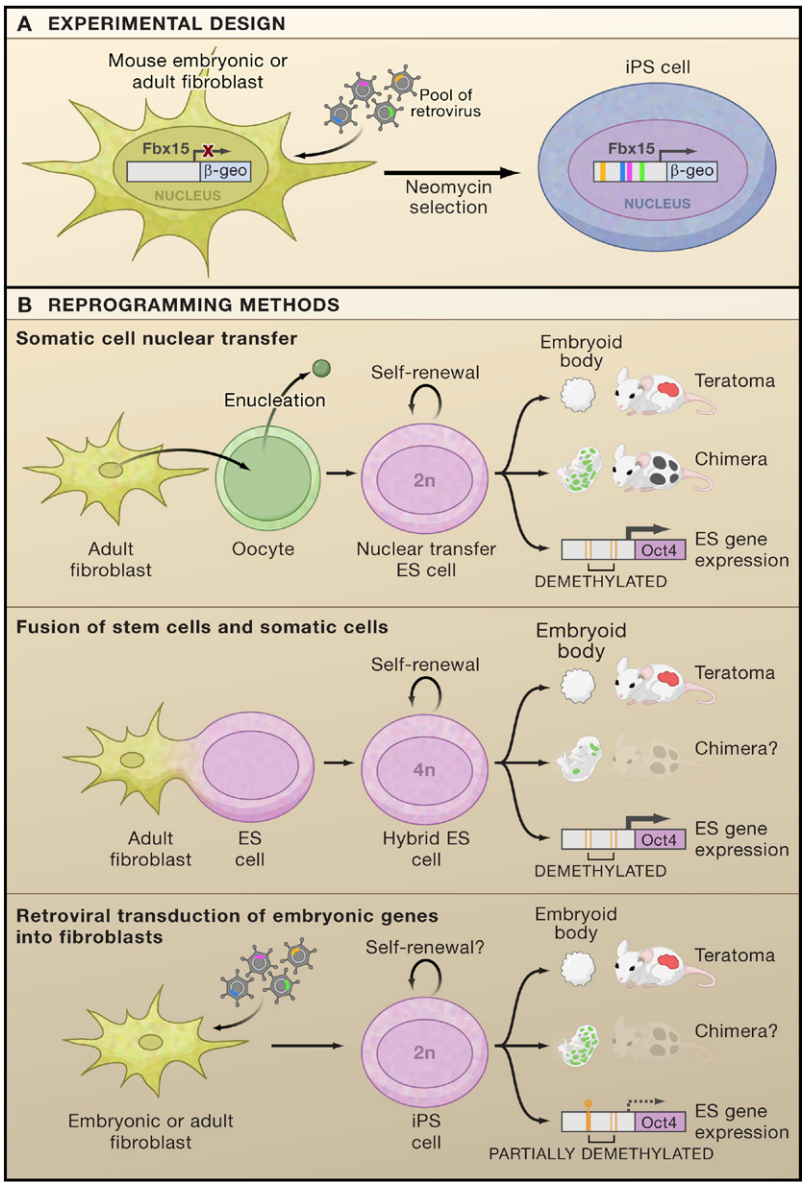

Figure 1. Reprogramming Differentiated Somatic Cells

(A) Embryonic and adult mouse fibroblasts expressing a selectable marker (β-geo) driven by an ES cell-specific promoter (*Fbx15*) are transduced with retroviruses encoding different candidate reprogramming factors. After selection with the antibiotic neomycin, drug-resistant β-galactosidase-positive cells are identified (Takahashi and Yamanaka, 2006). These cells, called iPS (induced pluripotent stem cells), have many of the characteristics of embryonic stem cells.

(B) Methods of nuclear reprogramming and their outcomes. (Top) In somatic cell nuclear transfer, a somatic cell nucleus is introduced into an enucleated oocyte and used to produce mouse ES cells. These nuclear transfer ES cells are completely reprogrammed: they self-renew, are pluripotent (they form embryoid bodies and teratomas), can contribute to all germ layers of mouse chimeras, and express a full complement of ES-specific genes. (Middle) Fusion of ES cells and somatic cells is shown. A somatic cell is fused with an ES cell, leading to the reprogramming of the somatic cell's nucleus. Although these fused hybrid cells show self-renewal, are pluripotent, and express the normal complement of ES-specific genes, the ability to test their contribution to chimeras remains difficult because of their tetraploid chromosome complement. (Bottom) Transduction of embryonic and adult mouse fibroblasts with retroviral vectors encoding embryonic transcription factors (Takahashi and Yamanaka, 2006). Although the transduced fibroblasts are pluripotent, they only contribute to mouse chimeras up to E13.5 (no live chimeric pups have been obtained). Further, the transduced fibroblasts are not completely reprogrammed, exhibiting incomplete reactivation of ES-specific genes and only partial demethylation of the *Oct3/4* locus.

Sox2, *c-Myc*, and *Klf4*. The resulting "iPS-MEF4" cells had morphological and growth characteristics similar to iPS-MEF10 cells. In additional experiments, the introduction of *Oct3/4*, *c-Myc*, and *Klf4* proved to be the only combination of three genes that could give rise to rapidly proliferating, drug-resistant cell lines. However, further experiments demonstrated that these iPS-MEF3 cells differed substantially from their counterparts that also expressed *Sox2*. Importantly, when tail-tip fibroblasts from adult mice were transduced with pools of viruses encoding the four cDNAs, the authors were able to isolate ES cell-like "iPS-TTF" cells, indicating that adult somatic cells could also be reprogrammed by this methodology.

These observations raise the question: Do the reprogrammed iPS cells have the salient features of authentic ES cells? IPS-MEF3, 4, and 10 cells all possessed the immortal growth characteristics of genuine, self-renewing ES cells. These iPS cells were also able to form embryoid bodies in vitro and teratomas in vivo. In these assays iPS-MEF10 and iPS-MEF4 cells, but not iPS-MEF3 cells, were able to differentiate into a variety of distinct cell types, demonstrating their developmental potential. Most remarkably, when iPS-TTF cells were injected into mouse blastocysts they contributed widely to diverse tissues in chimeric embryos recovered as late as embryonic day 13.5. Together, these results suggest that Takahashi and Yamanaka (2006) have successfully reprogrammed terminally differentiated cells to a pluripotent state.

Several observations, however, indicate that iPS cells are similar but not identical to ES cells. First, the absence of any contribution from iPS-derived cells to postnatal animals following blastocyst injection suggests that the cells have a limited capacity to stably integrate into normal tissues in vivo. Second, although rare iPS clones showed expression patterns of known ES-specific genes that were very similar to controls, a substantial degree of clone-to-clone variation was observed. Some clones failed to reactivate a number of the genes assayed, and notably none were found to express ES cell-associated transcript 1 (*Ecat1*). Transcriptional profiling experiments revealed that although the iPS cells clustered more closely to the ES cells than they did to their parental fibroblasts, they still possessed a distinct gene expression signature. Third, DNA methylation of the *Oct3/4* promoter and the posttranslational modification of histones positioned there suggested that the iPS cells are caught in an epigenetic state that is intermediate between their somatic origins and fully reprogrammed ES cells (Figure 1B, bottom). In summary, the nuclear reprogramming observed by introduction of transcription factors into somatic cells is substantial, but it differs from the more complete reprogramming that is observed after transfer of nuclei from somatic cells into oocytes (Figure 1B, top) or after fusion of somatic cells with ES cells (Figure 1B, middle). Clearly, an important question remains: Are these cells in fact trapped in an intermediate state between somatic cells and ES cells, or are they actually some other pluripotent cell type, such as embryonic carcinoma (EC) cells?

Takahashi and Yamanaka's (2006) observations raise other intriguing questions. If expression of the virally encoded transgenes is constitutive, as the authors suggest, how do the iPS cells begin to undergo differentiation? Could introduction of additional cDNAs result in cells that would be more fully reprogrammed? Would more prolonged culture lead to a completion of reprogramming and silencing of the viral transgenes? How general is this approach? Could this strategy be used to de-differentiate a variety of somatic cell types, including human cells? Of particular interest is the stability of the reprogrammed state. If reactivation of embryonic genes and epigenetic reprogramming is incomplete, can the iPS cells maintain their undifferentiated state without continued expression of the viral transgenes? This question is particularly pressing given the incomplete demethylation of the *Oct3/4* promoter and the low level of transcription originating from the endogenous alleles of *Oct3/4* and *Sox2*.

Another point of curiosity is how rarely iPS cells are recovered after retroviral cotransduction. Quantitative transduction experiments seemed to rule out the possibility that the infrequent appearance of these cells is caused by low cotransduction efficiency. It seems plausible, as the authors suggest, that there is a small range of expression levels for each of the factors that can lead to reprogramming and only rarely does a cell receive the proper retroviral dosage. Another possibility is that the cells being successfully reprogrammed are actually progenitors rather than the terminally differentiated fibroblasts that constitute the majority of cells in the cultures.

Nevertheless, the surprising fact that *any* pluripotent cells could be recovered by introducing embryonic transcription factors into fully differentiated somatic cells provides an additional perspective on the regulation of a cell's developmental identity. Just as overexpression of *MyoD* alone leads to the upregulation of muscle gene products and the induction of muscle characteristics in fibroblasts (Weintraub et al., 1989), Takahashi and Yamanaka's (2006) work lends further credence to the notion that perturbing a transcriptional network at a limited number of nodes can force it from one metastable state to another. Whether the reprogramming activity of this combination of genes results solely from the regulation of downstream targets at the transcriptional level, however, awaits further analysis.

What are the functions of the oncogenes *c-Myc* and *Klf4*? Although c-Myc is known to increase the expression of a number of genes important for proliferation and self-renewal (telomerase perhaps being one of the most important) (Cartwright et al., 2005), it may also have functions beyond direct transcriptional regulation. The large number

of predicted c-Myc binding sites in the genome and its association with histone acetyltransferase complexes prompted the authors to speculate that c-Myc may induce global histone acetylation (Fernandez et al., 2003), perhaps allowing Oct3/4 and Sox2 to bind to otherwise inaccessible sites. Unlike c-Myc, the role of Klf4 in reprogramming is more likely to be strictly one of transcriptional control. Its key contribution to this process is probably to downregulate the transcription of *p53* (Rowland et al., 2005), which is known to regulate expression of *Nanog* (Lin et al., 2005). Repression of *p53* also inhibits *c-Myc*-induced apoptotic pathways (Zindy et al., 1998), suggesting that *c-Myc* and *Klf4* may act reciprocally and that a finely tuned balance between them could be crucial for successful reprogramming.

Not only does the work of Takahashi and Yamanaka (2006) lend important insight into the molecular nature of reprogramming and pluripotency, it represents a significant step toward a rational approach for generating patient-specific ES cell lines that could be used either as a source of autologous tissue for transplantation or for modeling different diseases. This method is encumbered by neither the logistical constraints nor the societal concerns presented by somatic cell nuclear transfer. However, practical application of this approach still requires answering questions concerning the incomplete nature of the reprogramming observed, the constitutive expression of the transgenes, and the therapeutic utility of cells modified with known oncogenes and oncogenic viral vectors.

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Improving Synaptic Function in a Mouse Model of AD

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Memory loss is an early symptom of Alzheimer's Disease (AD). The findings of Gong et al. (2006) now indicate that enhancing the activity of UCH-L1, a ubiquitin hydrolase, alleviates the synaptic dysfunction and memory loss associated with a mouse model of AD. This work also raises the question of what role UCH-L1 might play in other diseases involving protein aggregation, such as Parkinson's Disease.

Understanding the molecular mechanism of memory is one of the most compelling and complex challenges for the next generation of scientists. In a paper that appears in this issue, Arancio and colleagues identify a protein that may participate in both normal memory formation and in the type of memory loss characteristic of early AD: the enzyme ubiquitin C-terminal hydrolase of the L1 type (UCH-L1) (Gong et al., 2006). They show that administration of a UCH-L1 fusion protein to supplement endogenous UCH-L1 has a protective effect on memory loss in a mouse model of AD. Although the authors propose that this effect is mediated by a process involved in normal memory, there is some evidence that UCH-L1 may also be involved in a protective path-